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# Uncovering a novel mechanism whereby NK cells interfere with glioblastoma virotherapy

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Despite initial promising results, the success of clinical trials testing oncolytic viruses in glioblastoma patients has been limited. Innate immunity appears to be one among several barriers against successful viral oncolysis. Recent findings suggest a mechanism by which natural killer cells limit the efficacy of oncolytic viruses via natural cytotoxicity receptors.

Despite years of work aimed at improving the prognosis of glioblastoma (GBM) patients, overall survival has remained largely unchanged. Novel approaches against this dreadful disease encompass the use of targeted anticancer agents to inhibit aberrant signaling pathways, anti-angiogenic agents, immunotherapy and virotherapy. In the latter setting, non-replicating viral vectors are used either to introduce exogenous transgenes or to promote antitumor immune responses. An alternative strategy for virotherapy relies on replicating oncolytic viruses that selectively infect tumor cells, eventually resulting in their lysis.

Phase I clinical trials studying oncolytic virotherapy in GBM patients have demonstrated the safety of this approach.<sup>1</sup> However, preliminary indications of efficacy have been disappointing. Thus, efforts have been dedicated at identifying barriers that would reduce the efficacy of oncolytic viruses as well as at designing second-generation viruses that can circumvent such impediments. Examples of processes that interfere with virotherapy include, but are not limited to, the interferon response that normally follows viral infection, the viral clearance mediated by the innate immune system, and the blockage of viral dissemination within the tumor as mediated by the extracellular matrix.<sup>2</sup>

The deleterious impact of innate immunity in virotherapy was first identified in preclinical animal studies demonstrating that the combination of cyclophosphamide (CPA) with an oncolytic herpes simplex virus (oHSV) significantly improves survival.<sup>3</sup> Immunohistochemical studies of tumor-bearing rats treated with CPA and oHSV unveiled a significant reduction in the number of macrophages, microglial cells, and natural killer (NK) cells within the tumor microenvironment, which correlated with increased viral replication.<sup>4</sup> Additionally, the efficacy of CPA combined with oHSV was improved in mice pretreated with clodronate liposomes (which deplete macrophages) as well as in animals genetically deficient for interferon  $\gamma$ .<sup>4,5</sup>

As they are endowed with both antiviral and antitumor functions, at least hypothetically NK cells may either inhibit the efficacy of virotherapy or promote additional degrees to tumor-cell killing, resulting in improved antineoplastic effects. Using the vesicular stomatitis virus (VSV) for the treatment of hepatocellular carcinoma, Altomonte et al. demonstrated that the depletion of NK cells enhances viral efficacy and increases overall survival.<sup>6</sup> A subsequent study was based on a second generation oncolytic VSV encoding a chemokine-binding protein that limits NK and NKT cell intratumoral infiltration,

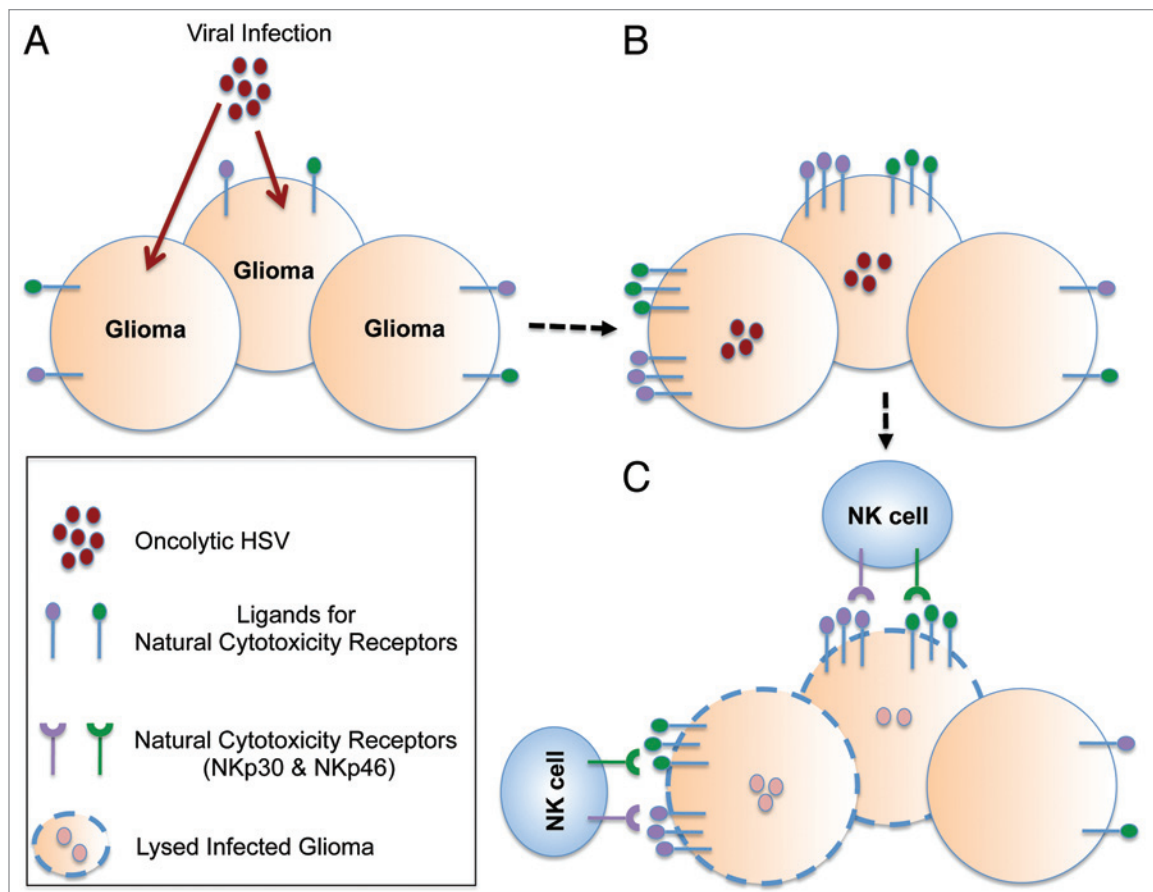
resulting in improved efficacy.<sup>7</sup> While these findings suggest that NK cells constitute an initial barrier to viral oncolysis, several groups have highlighted the necessity of harnessing the antitumor properties of these cells to achieve tumor clearance.<sup>8</sup> In this context, by using both xenograft and syngeneic mouse glioma models Alvarez-Breckenridge et al. have recently demonstrated the relevance of NK cells following oHSV infection (Fig. 1).<sup>9</sup> Indeed, following the intracranial inoculation of oHSV, NK cells were recruited into the brain and manifested an activated phenotype. In addition, NK cells were found to mediate the inflammatory cytokine and chemokine milieu that stems from viral infection while coordinating the activation of macrophage and microglial cells. Confirming the functional relevance of these findings, the survival of mice bearing either U87dEGFR glioma xenografts or 4C8 murine gliomas increased upon the depletion of NK cells prior to oHSV infection. These findings suggest that—presumably owing to their antiviral functions—NK cells play an important role in limiting the efficacy of oHSV-based therapeutic approaches against GBM.

To further understand the mechanisms underlying these observations, NK cell-mediated cytotoxicity was determined in vitro by co-culturing human NK cells with

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**Figure 1.** Deleterious role of natural killer cells in virotherapy. (A) Glioma cells normally express ligands for the natural killer (NK) cell natural cytotoxicity receptors NKp30 and NKp46. (B and C) Upon infection by oncolytic herpes simplex virus (oHSV), these ligands are overexpressed, correlating with a temporal increase in NK cell activation within the tumor microenvironment. NK cells kill oHSV-infected glioma cells via NKp30 and NKp46, in turn resulting in decreased viral titers and limiting the efficacy of viral oncolysis.

distinct human glioma cell lines. Of note, NK cell-mediated killing was significantly enhanced when tumors were initially infected with oHSV. By means of a panel of blocking antibodies, natural cytotoxicity receptors, notably NKp30 and NKp46, were identified as critical mediators of this response, which was paralleled by an increased expression on the tumor cell surface of NKp30 and NKp46 ligands. In line with these findings, intracranially inoculated oHSV exhibited elevated rates of replication in NKp46-deficient glioma-bearing mice, and increased the survival of these animals to higher extents than that of wild-type mice.

The study by Alvarez-Breckenridge et al. is the first to provide a potential mechanism whereby NK cells constitute an initial barrier to oHSV-based therapy (Fig. 1). Future clinical trials will need

to assess if these preclinical findings can be translated into a clinical setting. In particular, it will be important to determine if human NK cells infiltrate the tumor microenvironment upon infection by oncolytic viruses; to isolate tumor-infiltrating and circulating NK cells and determine their functional profile; and to correlate NK cell, macrophagic and microglial responses with the survival of patients subjected to virotherapy. From a preclinical perspective, future studies will have to identify the NKp30 and NKp46 ligands that are upregulated in response to oHSV infection, as the blockage of receptor-ligand interactions may have important therapeutic implications.

While the work by Alvarez-Breckenridge et al. highlights a deleterious role of NK cells in the context of virotherapy, the importance of antitumor immunity should

not be overlooked. Indeed, to achieve successful therapeutic responses upon virotherapy, there must be a delicate balance between the antiviral and antitumor immunity. Mathematical modeling has proposed that shortly after infection, the innate immune system should be suppressed to achieve multiple rounds of viral replication.<sup>10</sup> Once a sufficient viral load is attained to achieve tumor killing, antitumor immune effectors including NK cells may constitute a valuable partner for tumor clearance. The challenge for future studies will be to identify both a mechanism and a time point for switching from the suppression of antiviral immunity to the activation of antitumor immune responses.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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